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THYROXINE STIMULATION OF AMINO ACID INCORPORATION INTO PROTEIN INDEPENDENT OF ANY ACTION ON MESSENGER RNA SYNTHESIS

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Recent studies on the biochemical basis of the physiological actions of the thyroid hormone have directed attention to its action on protein biosynthesis.[1, 2] The administration of L-thyroxine to normal rats stimulates the rate of amino acid incorporation into protein assayed *in vitro* in cell-free liver preparations.[1, 2] Thyroid-

ectomy results in a reduction in this rate[2, 3] which can be raised toward normal by thyroid hormone replacement therapy.[3, 4]

The thyroxine effect on protein synthesis appears to be physiologically significant. Amino acid incorporation into protein *in vivo* is increased in thyroxine-treated animals in the liver, heart, and kidney,[5] organs which respond to thyroxine with increased oxygen consumption.[6] In the brain, spleen, and testis, organs in which oxygen consumption is unchanged in hyperthyroidism,[6] there is no effect on amino acid incorporation into protein.[5] As originally suggested by Sokoloff and Kaufman,[1] the thyroxine effect on metabolic rate appears to be secondary to its effect on protein biosynthesis. Blockade of protein biosynthesis and, therefore, also the thyroxine stimulation of protein biosynthesis by puromycin interrupts the thyroxine stimulation of oxygen consumption and almost immediately restores the increased basal metabolic rate of thyrotoxic animals to the level of normal or puromycin-treated euthyroid controls.[7] Inhibition of protein biosynthesis by puromycin or actinomycin D in hypothyroid rats has also been reported to prevent the stimulation of metabolic rate by simultaneously administered triiodothyronine.[8]

The addition of thyroxine in low concentrations directly to the incubation mixture also stimulates amino acid incorporation into protein in cell-free rat liver preparations.[1, 2] This finding of an *in vitro* effect of the hormone has greatly facilitated studies of its mechanism of action. The effect has been found to be a true stimulation and not merely a preservation of the initial rate.[2] Mitochondria and an oxidizable substrate are essential requirements for the effect; when these components of the reaction mixture are replaced by an alternative ATP generating system, no stimulation by thyroxine is observed.[2, 9] The stimulation has been localized to the step in protein biosynthesis involving the transfer of sRNA-bound amino acid to microsomal protein,[9, 10] and it is not secondary to an effect on the generation of GTP, ATP, or reduced glutathione, the only cofactors known to influence or to be required in this step.[9] The role of the mitochondria appears to be in a preliminary reaction with thyroxine to produce a still unidentified factor which is responsible for the stimulation of the transfer step.[9] The stimulation is not the result of an increase in permeability of the microsomal membrane since it occurs with ribonucleoprotein particles as well.[11] The thyroxine stimulation occurs only when the rate of amino acid incorporation into protein is limited by the ribosomal concentration; when ribosomes are present in excessive or inhibitory amounts, the action of thyroxine is to enhance the inhibition.[11] The thyroxine effect on amino acid incorporation into protein appears, therefore, to result from some action on ribosomes which increases their activity.

Widnell and Tata[12] have recently reported that thyroid hormone administration *in vivo* increases nuclear RNA polymerase activity before it stimulates amino acid incorporation into protein. They therefore proposed that increased messenger RNA production is the mechanism by which thyroid hormone stimulates amino acid incorporation into protein. Such a mechanism is consistent with current concepts of the genetic control of protein biosynthesis.[13] The present studies demonstrate, however, that thyroxine stimulates amino acid incorporation into protein independently of any action on DNA-dependent RNA polymerase activity or messenger RNA synthesis and can, in fact, stimulate synthetic polyribonucleotide-directed amino acid incorporation into polypeptide.

Materials and Methods.—Chemicals and enzymes: Compounds obtained from commercial sources were of the highest grade of purity available. DL-Leucine-1-C^{14}, DL-valine-1-C^{14}, and DL-phenylalanine-1-C^{14} were obtained from either the Nuclear-Chicago Corp. or the New England Nuclear Corp. AMP-8-C^{14} was obtained from Schwarz BioResearch, Inc. Creatine kinase (E.C. 2.7.3.2) was purchased from C. F. Boehringer and Soehne Gmbh., Mannheim, West Germany. Ribonuclease A (E.C. 2.7.7.16) and deoxyribonuclease (E.C. 3.1.4.5) were obtained from Sigma Chemical Co. and Worthington Biochemical Corp., respectively. Actinomycin D was the gift of Merck, Sharpe and Dohme Research Laboratories. Polyuridylic acid (poly U) was purchased from the Miles Chemical Co. All other chemicals and compounds were the same as those previously described.[1,2,9]

Animals: Normal Osborne-Mendel male rats weighing between 80 and 150 gm were used in all experiments. The animals were maintained on Purina laboratory chow and tap water but were fasted at least 17 hr immediately prior to killing.

Preparation of homogenates and cell fractions: Liver homogenates were prepared fresh for each experiment. Homogenization procedures were the same as those previously described.[1,2] For experiments in which all flasks contained the complete system, i.e., mitochondria, microsomes, and cell sap, fractionation and reconstitution of the crude homogenates were carried out according to Procedure A of Sokoloff and Kaufman.[2] In experiments in which the mitochondria and oxidizable substrate were replaced by a creatine phosphate-ATP generating system (Table 2), their Procedure C was employed.

Nuclei were isolated from rat liver by the method of Sporn, Wanko, and Dingman.[14]

Assay methods: Incubation conditions were the same as those previously described.[1,2] The components of the reaction mixtures are described in the legends to the tables. The reactions were terminated by the addition of 5 ml of ice-cold 0.25 *M* sucrose solution containing 1 mg of the nonradioactive species of the C^{14}-labeled compound used in the assay per ml. The mitochondria and nuclei, if present, were then removed by centrifugation for 15 min at 12,800 × *g* in the Servall refrigerated centrifuge, and the protein and RNA in the 12,800 × *g* supernatant fluid were precipitated by the addition of an equal volume of 12% trichloroacetic acid. The precipitated protein was then purified and assayed for specific activity as previously described.[1,2]

In the experiments in which the specific activity of the RNA was measured (Table 1), the precipitate containing the protein and RNA was washed three times with 3% perchloric acid, twice with 0.06% perchloric acid, once with 70% ethanol, and finally suspended in 1 ml of a solution containing 0.1 *M* NaCl, 0.05 *M* potassium phosphate buffer, pH 7.2, and 0.0025 *M* EDTA. The RNA was then isolated from the suspension by phenol extraction.[15] Specific activity of the RNA was determined by measurement of both the absorbancy at 260 mμ and the radioactivity in appropriately diluted aliquots of the RNA solution. Purity of the RNA was checked by measurement of the ratio of absorbancies at 280 and 260 mμ. Radioactivity was measured in a liquid scintillation counter, and the counting rates of all samples were individually calibrated by means of C^{14} internal standards. Sufficient counts were collected to obtain a coefficient of variation of less than 1%.

Results.—Effects of thyroxine on RNA synthesis: The possibility that the thyroxine effect on amino acid incorporation into protein might be secondary to an effect on RNA synthesis was examined in experiments in which the effects of 6.5×10^{-5} *M* thyroxine on AMP-8-C^{14} incorporation into RNA and DL-leucine-1-C^{14} incorporation into protein were simultaneously compared. The results failed to provide any evidence to support this possibility (Table 1). Thyroxine stimulated amino acid incorporation into protein in the absence of any significant effects on the incorporation of AMP-8-C^{14} into RNA.

Effects of added nuclei on thyroxine stimulation of amino acid incorporation into protein: The results of the experiments illustrated in Table 1 offered no support for a thyroxine effect on RNA synthesis, but neither did they conclusively exclude the possibility of an effect on a specific small pool of RNA, for example, messenger RNA, the specific activity of which might be diluted out by the bulk of the RNA present in the incubation system. Although the procedure for the preparation of the cell

TABLE 1

EFFECTS OF L-THYROXINE ON INCORPORATION OF AMP-8-C^{14} INTO RNA AND DL-LEUCINE-1-C^{14} INTO MICROSOMAL PROTEIN

Assay	Incubation time (min)	Specific Activity: Control	Specific Activity: +L-Thyroxine	Thyroxine Effect: Δ	Thyroxine Effect: %
		mμmoles AMP-8-C^{14} per A_{260} unit of RNA			
AMP-8-C^{14} incorporation into RNA	5	0.09	0.10	+0.01	+11
	10	0.15	0.15	0.00	0
	20	0.21	0.14	−0.07	−33
		cpm/mg of protein		Δ	%
DL-Leucine-1-C^{14} incorporation into protein	20	43.0	63.5	+20.5	+48

The components of the reaction mixtures in μmoles were as follows: sucrose, 158; potassium phosphate buffer, pH 7.4, 20; $MgCl_2$, 5; GTP, 0.25; sodium DL-β-hydroxybutyrate, 50; nonradioactive AMP or AMP-8-C^{14} (specific activity = 0.64 μc per μmole), 5; and nonradioactive DL-leucine or DL-leucine-1-C^{14} (specific activity = 5.4 μc per μmole), 0.8. Each experimental flask received sufficient sodium L-thyroxine dissolved in 0.1 ml of 0.01 *N* NaOH to achieve a final concentration of 6.5×10^{-5} *M*; control flasks received equivalent amounts of NaOH alone. In addition, each flask received 0.45 ml of homogenate prepared by Procedure A of Sokoloff and Kaufman,[2] which contained mitochondria and microsomes equivalent to the yield from 200 mg and cell sap equivalent to the yield from 30 mg of fresh liver. The reaction mixture was brought to a final volume of 1.7 ml with water. Incubation time at 37°C was as indicated in the table. All other incubation procedures were as previously described.[2]

fractions employed in these studies included a step for the removal of nuclei, there was no assurance of the complete absence of nuclear contamination. It was, therefore, still possible that the thyroxine stimulation of amino acid incorporation into protein was secondary to an effect on nuclear RNA polymerase activity. The possibility was also considered that the mitochondrial requirement for the thyroxine effect on amino acid incorporation previously observed[2, 9] reflected only a requirement for nuclei contaminating the mitochondrial fraction. Experiments were, therefore, carried out in which the level of nuclear RNA polymerase activity in the incubation system was progressively increased by the addition of graded amounts of rat liver nuclei. Nuclei prepared by the same method have been shown to exhibit RNA polymerase activity *in vitro*[12] and to contain RNA with messenger activity.[16] As can be seen in Table 2, the addition of nuclei to the com-

TABLE 2

EFFECTS OF ADDED NUCLEI ON THE THYROXINE EFFECT ON DL-LEUCINE-1-C^{14} INCORPORATION INTO MICROSOMAL PROTEIN IN THE PRESENCE AND ABSENCE OF MITOCHONDRIA AND OXIDIZABLE SUBSTRATE

System	Nuclear addition per flask (fresh liver weight equivalent), mg	Control	+L-Thyroxine	L-Thyroxine Effect: Δ cpm/mg	L-Thyroxine Effect: %
		cpm/mg of protein			
Complete	0	92.3	167.7	+75.4	+82
	35	110.6	159.4	+48.8	+44
	70	115.7	171.5	+55.8	+48
	140	109.3	162.9	+53.6	+49
Minus mitochondria, minus DL-β-hydroxybutyrate, plus creatine phosphate, plus creatine kinase	0	90.0	85.5	− 4.5	− 5
	35	94.6	87.0	− 7.6	− 8
	70	76.3	79.1	+ 2.8	+ 4
	140	84.2	83.0	− 1.2	− 1

Homogenate fractions were prepared by Procedure C of Sokoloff and Kaufman.[2] The complete system contained the same components as the reaction mixtures containing the DL-leucine-1-C^{14} described in Table 1, including 0.15 ml of the mitochondrial suspension and 0.30 ml of the microsomal-cell sap mixture added separately. The contents of the flasks without mitochondria and DL-β-hydroxybutyrate were identical, except that the mitochondrial suspension was replaced by 0.15 ml of 0.25 *M* sucrose solution, and the DL-β-hydroxybutyrate was replaced by 40 μmoles of creatine phosphate and 0.25 mg of creatine kinase contained in an equivalent volume. In addition, each flask received 0.2 ml of a solution of 0.32 *M* sucrose, 0.001 *M* $MgCl_2$, and 0.0008 *M* potassium phosphate buffer, pH 6.8, containing the quantity of nuclei indicated in the table. The L-thyroxine concentration was 6.5×10^{-5} *M*. Incubation time at 37°C was 25 min. All other incubation conditions were the same as those in Table 1.

plete system containing mitochondria and an oxidizable substrate may increase slightly the rate of amino acid incorporation into protein but never sufficiently to replace the thyroxine effect. Thyroxine continues to stimulate even in the presence of optimal or greater than optimal amounts of nuclei. Furthermore, when the mitochondria and oxidizable substrate are replaced by a creatine phosphate-ATP generating system, there is no thyroxine effect, even in the presence of the added nuclei. There is, in fact, no indication that the rate of amino acid incorporation into protein is limited by nuclear RNA polymerase activity. These results offer further evidence that the thyroxine stimulation of amino acid incorporation into protein is not secondary to an effect on nuclear RNA polymerase activity and that the mitochondrial requirement is not a reflection of a possible nuclear contamination of the mitochondrial fraction.

Thyroxine stimulation of amino acid incorporation into protein in presence of inhibitors of DNA-dependent RNA polymerase activity: Inhibition of DNA-dependent RNA polymerase activity by actinomycin D or DNAase had no effects on either the control rate of amino acid incorporation into protein or the thyroxine effect (Table 3). In contrast, RNAase markedly inhibited both. The results were essentially the

TABLE 3

EFFECTS OF INHIBITION OF DNA-DEPENDENT RNA POLYMERASE ON THE L-THYROXINE STIMULATION OF DL-LEUCINE-1-C^{14} INCORPORATION INTO PROTEIN

Inhibitors	Control cpm/mg of	+L-Thyroxine protein	L-Thyroxine Effect Δ cpm/mg	%
No preincubation:				
None	66.3	90.5	+24.2	+36
Actinomycin D	67.6	84.2	+16.6	+25
DNAase	64.8	90.0	+25.2	+39
RNAase	15.0	10.2	− 4.8	−32
Following 5 min preincubation:*				
None	50.1	83.2	+33.1	+66
Actinomycin D	47.9	85.2	+37.3	+78
DNAase	43.3	78.2	+34.9	+81
RNAase	14.9	12.9	− 2.0	−13

* The complete system including the inhibitors was preincubated at 37°C for 5 min before the addition of the DL-leucine-1-C^{14} and the start of the reaction.

The components of the reaction mixtures were the same as those described for the assay of DL-leucine-1-C^{14} incorporation into protein in Table 1, except that the specific activity of the DL-leucine-1-C^{14} was 5.7 μc per μmole, and 10 μg of actinomycin D, 10 μg of DNAase, or 0.1 mg of RNAase A were added as indicated in the table. The L-thyroxine concentration was 6.5×10^{-4} *M*. Incubation time at 37°C was 25 min.

same whether or not the reaction mixtures were first preincubated in the presence of the inhibitors for 5 min at 37°C. The same solutions of these inhibitors at the same concentrations effectively inhibited messenger RNA synthesis in an *E. coli* system. The results of these experiments demonstrate that the rate of amino acid incorporation into protein in the rat liver system used in these studies is not limited by DNA-dependent RNA polymerase activity and that thyroxine still stimulates equally well even in the relative absence of this enzymatic activity. The thyroxine stimulation of amino acid incorporation into protein cannot, therefore, be mediated by an effect on the synthesis of messenger RNA.

Thyroxine stimulation of poly U-directed DL-phenylalanine-1-C^{14} incorporation into polyphenylalanine: Thyroxine also stimulates synthetic messenger RNA-directed incorporation of amino acids into polypeptides. In Table 4 are illustrated the results of experiments in which poly U was added to incubation mixtures containing liver preparations from normal rats (Expt. 1) and rats pretreated with actinomy-

TABLE 4

EFFECTS OF L-THYROXINE ON POLY U-DIRECTED PHENYLALANINE INCORPORATION INTO POLYPHENYLALANINE

Expt. no.	C^{14}-amino acid added	Poly U addition per flask (μg)	Incorporation into Protein and Polyphenylalanine: Control cpm in protein and polyphenylalanine per mg	Incorporation into Protein and Polyphenylalanine: Thyroxine cpm in protein and polyphenylalanine per mg	Thyroxine Δ cpm/mg	Effect %	Incorporation into Polyphenylalanine: Control cpm in polyphenylalanine per mg	Incorporation into Polyphenylalanine: Thyroxine cpm in polyphenylalanine per mg	Thyroxine Effect Δ cpm/mg	Thyroxine Effect %
1*	DL-Phenylalanine-1-C^{14}	0	107	137	+30	+28	0	0	–	—
		100	123	175	+52	+42	16	38	+22	+138
		200	135	184	+49	+36	28	47	+19	+ 68
		300	138	192	+54	+39	31	55	+24	+ 77
	DL-Valine-1-C^{14}	0	65	93	+28	+43	—	—	—	—
		200	51	92	+41	+80	—	—	—	—
2†	DL-Phenylalanine-1-C^{14}	0	188	202	+14	+ 7	0	0	—	—
		100	219	275	+56	+26	31	73	+42	+135
		200	218	251	+33	+15	30	49	+19	+ 63
		300	200	270	+70	+35	12	68	+56	+467
	DL-Valine-1-C^{14}	0	88	100	+12	+14	—	—	—	—
		200	78	100	+22	+28	—	—	—	—

* Homogenate prepared from normal rat liver.

† Homogenate prepared from liver of rat given 15 μg actinomycin D per 100 gm of body weight intraperitoneally 42 hr previously.

Components of the reaction mixtures and incubation conditions were the same as those described for the assay of DL-leucine-1-C^{14} incorporation into protein in Table 1 with the following exceptions: the $MgCl_2$ addition per flask was 6 μmoles; poly U was added to the flasks in the amounts indicated; each flask received 1 μmole of either nonradioactive DL-phenylalanine or DL-phenylalanine-1-C^{14} (specific activity = 5.0 μc per μmole), and 0.8 μmole of either nonradioactive DL-valine or DL-valine-1-C^{14} (specific activity = 3.05 μc per μmole). Only one radioactive amino acid was added per flask as indicated in the table. The homogenates were prepared by Procedure A of Sokoloff and Kaufman,[3] except that 10^{-4} *M* EDTA was added to the homogenizing and resuspending medium. The thyroxine concentration was 6.5×10^{-5} *M*. Incubation time at 37°C was 25 min. The DL-phenylalanine-1-C^{14} incorporation into polyphenylalanine was calculated by subtraction of the base-line incorporation of DL-phenylalanine-1-C^{14} into protein in the absence of poly U from the specific activity of the combined protein and polyphenylalanine obtained in the presence of poly U. The difference was taken to represent the quantity of polyphenylalanine-C^{14} per mg of combined protein and polyphenylalanine.

cin D (Expt. 2). The actinomycin D pretreatment was employed in an attempt to deplete the natural messenger RNA content of the tissue *in vivo* and thus enhance the response of the preparation to added poly U. However, in agreement with the recent findings of Revel and Hiatt,[17] no significant effects on base-line amino acid incorporation into protein or sensitivity to poly U were observed with doses of actinomycin D varying from 15 μg to 240 μg per 100 gm of body weight administered intraperitoneally 16–42 hr prior to the killing of the animals. The actinomycin D pretreatment did, however, appear to lower the thyroxine stimulation of base-line amino acid incorporation into protein.

Poly U stimulated the incorporation of DL-phenylalanine-1-C^{14}, but the thyroxine stimulation was superimposed even on the maximal poly U effect. The effects of thyroxine and poly U appeared to be synergistic, each enhanced by the presence of the other. In fact, in occasional experiments the poly U effect was essentially negligible in the control flasks and evident only in the presence of thyroxine. Although thyroxine enhanced the poly U effect, it did not lower the optimal poly U concentration; the thyroxine effect, therefore, does not appear to be secondary either to the inhibition of a nuclease which degrades poly U or to the stimulation of the attachment of messenger RNA to ribosomes. These possibilities are, however, under further study.

The poly U effect was specific for phenylalanine; DL-valine-1-C^{14} incorporation was unaffected or even inhibited by poly U. The poly U-dependent DL-phenylalanine-1-C^{14} incorporation was assumed, therefore, to represent incorporation into polyphenylalanine, the polypeptide coded by poly U.[18] It can be seen in Table 4 that thyroxine stimulated the poly U-directed incorporation into polyphenylalanine even more so than the incorporation of amino acid into protein. The fact that thyroxine stimulates synthetic messenger RNA-directed amino acid incorporation is evidence that thyroxine stimulates protein biosynthesis independently of any action on the synthesis of natural messenger RNA.

Discussion.—The present studies include four different types of experiments, each of which provides independent evidence that thyroxine stimulates amino acid incorporation into protein independently of any action on messenger RNA synthesis. The addition of thyroxine *in vitro* to cell-free, normal rat liver homogenates results in a stimulation of amino acid incorporation into microsomal protein in the absence of any detectable effect on the incorporation of RNA precursors into RNA. The thyroxine stimulation of amino acid incorporation into protein is present even when nuclei containing RNA polymerase activity and messenger RNA are added in optimal amounts. Inhibition of DNA-dependent RNA polymerase activity by actinomycin D or DNAase has no effect on amino acid incorporation into protein or the thyroxine stimulation. Finally, thyroxine stimulates poly U-directed incorporation of phenylalanine into polyphenylalanine. The results of these studies suggest that there is a mechanism by which the thyroid hormone regulates the rate of protein biosynthesis independently of any action at the gene level.

These results are in disagreement with the mechanism of the thyroxine stimulation of protein biosynthesis proposed by Widnell and Tata.[12] These workers found that RNA polymerase activity in nuclei prepared from the livers of thyroidectomized rats is low, and that thyroid hormone administration to such animals raises the nuclear RNA polymerase activity toward normal before the effect on amino acid

incorporation into protein is observed. They therefore suggested that the effect of thyroid hormone on protein biosynthesis is secondary to an action at the gene level which stimulates messenger RNA synthesis. The change in protein synthesis observed by Tata and his associates[3, 12] in thyroid-deficient animals treated with replacement therapy is a clearly different phenomenon from the stimulation observed in this laboratory when thyroid hormone is administered to normal animals or added directly *in vitro* to cell-free preparations from normal animal tissues. There is no inherent difference in the amino acid-incorporating activities of microsomes isolated from normal rats and from rats treated *in vivo* with thyroxine. They exhibit a difference only when they are assayed in the presence of their corresponding mitochondria. Both types of microsomes incorporate amino acid into protein at the normal rate in the presence of normal rat liver mitochondria, and both are equally stimulated in the presence of mitochondria from the livers of thyroxine-treated rats.[1, 2] Thyroxine added *in vitro* stimulates amino acid-incorporating activity in normal rat liver microsomes only if mitochondria are present in the reaction mixture.[2, 9] Clearly, mitochondria are involved in the mechanism of the effect. On the other hand, microsomes from chronically thyroid-deficient rats have an inherently lower than normal amino acid-incorporating activity that persists even when assayed in the absence of mitochondria.[3, 4] Amino acid incorporation into protein in hypothyroid rat liver microsomes can also be stimulated by thyroxine added *in vitro* in the presence of mitochondria, but to a considerably lesser degree than in normal microsomes.[19] This degeneration of microsomal protein biosynthetic function in hypothyroidism can be reversed by thyroid hormone replacement therapy *in vivo*, but it is a delayed effect which appears after a latent period of approximately 35 hr.[3] Chronic thyroid deficiency appears then to result in an intrinsic degeneration in the microsomal protein biosynthetic machinery which can be restored to the normal state by replacement therapy. There appears to be no corresponding effect in the action of thyroid hormones in normal animals. This degenerative effect may, therefore, be a consequence of the disease state or a secondary cellular adaptation to the reduction in protein biosynthesis resulting from the removal of mitochondria-dependent thyroxine stimulation. Tata and his associates[3, 12] are studying the effects of thyroid hormone replacement therapy in thyroid-deficient rats. Since they do not include mitochondria in their assay system for amino acid incorporation into protein, they observe only the effects of the regeneration of the deficient microsomes. The regeneration is a delayed and probably secondary change which may, in fact, be dependent on increased messenger RNA synthesis. The mitochondria-dependent thyroxine stimulation of protein biosynthesis is likely, however, to be more specific and direct since it is the only one thus far observed when thyroxine is administered to normal animals or added *in vitro* to cell-free systems. It is the mitochondria-dependent thyroxine stimulation which the present studies have demonstrated to be independent of any action on messenger RNA synthesis.

Summary.—Thyroxine added *in vitro* to cell-free rat liver preparations stimulates amino acid incorporation into protein in the absence of any effect on the incorporation of RNA precursors into RNA. The thyroxine stimulation is superimposed on the effects of optimal or greater than optimal amounts of nuclei containing RNA polymerase activity and messenger RNA. Inhibition of DNA-dependent RNA

polymerase activity by actinomycin D and DNAase has no effect on the control rate of amino acid incorporation into protein or the thyroxine effect. Thyroxine also stimulates the rate of poly U-directed incorporation of phenylalanine into polyphenylalanine. These results are interpreted to indicate that thyroxine stimulates amino acid incorporation into microsomal protein independently of any action on DNA-dependent RNA polymerase activity or messenger RNA synthesis.

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